1777-Pos Board B687
Computer Simulations of how EFC F-BAR Domain Lattices Sculpt Membranes
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Biological cells are dynamically sculpted into compartments by cellular membranes. For example, in vitro experiments BAR domains induce tubule formation from lipid liposomes. Previous simulations showed how different lattice arrangements of N-BAR domains shape membrane into tubules (Yin, Arkhipov, and Schulten, 2009). Here we characterized, through 1-microsecond all-atom simulations, how F-BAR domains sculpt lipid membranes into tubes. For this purpose, a highly detailed, dynamic picture of 200-microsecond formation of membrane tubules by F-BAR domains is obtained. A coarse-grained (CG) model that accounts properties for F-BAR domains flexibility had been built. CG simulations of parallel rows of F-BAR domains on a DOPC/DOPS membrane show how extended-FCH F-BAR domains, in different lattice configurations, induce membrane curvature, with radii in the range of 30-100nm, in agreement with experimental observations.

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Dengue Virus Ensures its Fusion in Late Endosomes Using Compartment-Specific Lipids
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Many enveloped viruses invade cells via endocytosis and use different environmental factors as triggers for virus-endosome fusion that delivers viral genome into cytosol. Intriguingly, dengue virus (DEN), the most prevalent mosquito-borne virus that infects up to 100 million people each year, fuses only in late endosomes, while activation of DEN protein fusogen glycoprotein E is triggered already at pH characteristic for early endosomes. Are there any cofactors that time DEN fusion to virion entry into late endosomes? Here we show that DEN utilizes bis(monoacylglycero)phosphate (BMP), a lipid specific to late endosomes, as a co-factor for its endosomal acidification-dependent fusion machinery. Effective virus fusion to plasma- and intracellular- membranes, as well as to protein-free liposomes, requires the target membrane to contain anionic lipids such as bis(monoacylglycero)phosphate and phosphatidylserine. Anionic lipids act downstream of low-pH-dependent fusion stages and promote the advance from the earliest hemifusion intermediates to the fusion pore opening. To reach anionic lipid enriched late endosomes, DEN travels through acidified early endosomes but we found that low pH-dependent loss of fusogenic properties of DEN is relatively slow in the presence of anionic lipid-free target membranes. We propose that anionic lipid dependence of DEN fusion machinery protects it against premature irreversible restructuring and inactivation and ensures viral fusion in late endosomes, where the virus encounters anionic lipids for the first time during entry. Currently there are neither vaccines nor effective therapies for DEN and the essential role of the newly identified DEN-BMP interactions in viral genome escape from the endosome suggests a novel target for drug design.

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Fusogenic Activity of Annexins A1 and A5
Sung-Tae Yang, Eugenia Leikina, Kamran Melikov, Kozlov M. Michael, Leonid V. Chernomordik.
Annexins are a family of proteins that bind to anionic phospholipids in a calcium-dependent manner and have been implicated in a number of cell biological processes including intracellular fusion. Recently we found that extracellular annexins (A1 and A5) play important roles in myoblast fusion. In this study we explored fusogenic activity of these proteins in the absence of other proteins and found both A1 and A5 to aggregate phophatidylserine-containing liposomes and to induce lipid mixing between these liposomes. Annexins A1 and A5 drive fusion beyond early hemifusion stage, as evidenced by lipid mixing between the inner leaflets of liposomal membranes. The annexin-mediated vesicle aggregation and lipid mixing depended on the presence of calcium, anionic lipids and were inhibited by specific antibodies and, in the case of annexin A1, by a synthetic peptide derived from its N-terminal domain. Annexins A1 and A5 applied together were more effective in inducing fusion than either of the proteins on its own. This synergistic activity can be important in fusion between myoblasts since both of these proteins are present at myoblast surface under fusion conditions. Our results support the hypothesis that annexins directly mediate myoblast fusion by mechanisms similar to those discussed for viral and intracellular protein fusogens rather than regulate cell fusion via other proteins.

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Annexins and Dynamin in Myoblast Fusion
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Cell-to-cell fusion is a key stage in many developmental processes including fertilization and formation of bone, placenta, and muscles, as well as in mature organisms in muscle repair and in formation of multinucleated giant cells during inflammatory reactions. A major challenge in studying molecular mechanisms of membrane fusion is to isolate the actual fusion stage from the preceding stages that prime the cells to fusion. As a result, even for well characterized myoblast fusion in formation of muscle fibers, we still do not know the specific proteins that fuse lipid bilayers of two plasma membranes into one. In this study, we isolated fusion stage in myotube formation by murine myoblasts (C2C12 cells) labeled with different membrane and cytosolic probes by blocking myotube formation immediately prior to fusion with reversible hemifusion-inhibitor lysophosphatidylcholine. This approach accumulates cells at a ready-to-fuse stage and, thus, synchronizes fusion upon lifting the inhibitor. Isolation of the fusion stage allowed us to explore myoblast fusion pathway and identify annexins A1 and A5 as proteins that are present at cell surface at the time of fusion and play an important role in fusion. Based on the ability of these proteins to fuse liposomes (Yang et al.), we suggest that annexins directly mediate rather than regulate cell fusion. Annexin-dependent early stages of myoblast fusion are followed by expansion of fusion pores that we found to depend on cell metabolism and be blocked by dynasore and MitMAB, inhibitors of dynamin GTPase. We propose that annexins and dynamin are also involved in other cell-to-cell fusion processes and hope that better understanding of protein-lipid interactions underlying different stages of cell fusion will bring new ways of controlling membrane fusion in pathophysiology.

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Genetically Unmodified iPS Cells Generated by Microslit Confined Cell Fusion
Chi-cheng Fu.
Adult somatic cells can be used to derive induced pluripotent stem (iPS) cells by transduction of transcription factors. Although this technique shows great potential on various applications, its clinical applications are often hampered because of two main obstacles. Firstly, the reprogramming efficiency is generally very low (about 0.01-0.1%), and entire reprogramming process takes weeks. Second, most methods involved genome integration is skeptical in clinical applications because of the increasing chance to generate oncogenic cells. We are developing a method, called microslit-confined cell fusion, as shown in the Figure, to overcome these limitations. First, fibroblasts and stem cells are paired on each side of slits. Secondly, after elongation and contact, cells are fused together by an electrical pulse. Thirdly, reprogramming factors with other proteins in cytoplasm of stem cells diffuse to fused fibroblasts and direct them into pluripotent state. Finally, those partially fused entities will separate spontaneously after few days. These dynamics will be observed by time-lapse fluorescence microscopes. Our goals are to achieve 70% reprogramming efficiency and to complete the reprogramming process in 2 days. It can be 10000-times more efficient and 10-times faster than other gene integration free methods.